# Pharmacological rational of dry ripe fruit of *Aegle marmelos* L. as an anti-nociceptive agent in different painful conditions

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**Abstract**: The aim of study is to investigate central and peripheral analgesic effects of methanolic extract of dry ripe fruit of *Aegle marmelos* Linn. Corea (Am. Cr) by two methods, tail flick test and acetic acid induced writhing test at 100, 250 and 500mg/kg doses in animal models. Analgesic activity against tail flick test revealed that Am. Cr induced significant increase in latency period in dose dependent manner i.e. 65.38% at 100mg/kg, 395.37% at 250mg/kg ($p$<0.01) and 459.25% at 500mg/kg ($p$<0.01) body weight at 1hr after drug delivery while at 2hr effect decreased i.e. 61.53% at 100mg/kg, 161.11% ($p$<0.01) at 250mg/kg and 165.74% ($p$<0.01) at 500mg/kg but interestingly again there is an elongation in latency period at 3hr i.e. 106.15% at 100mg/kg dose, 251.85% ($p$<0.01) at 250mg/kg and 293.51% ($p$<0.05) at 500mg/kg respectively. The standard drug Diclofenac sodium at the dose of 5mg/kg continuously increased the latency period but less significantly as compared to the test substance i.e. 79.43%, 113.08% and 222.42% ($p$<0.05) respectively. Acetic acid induced writhing test produced highest significant activity at the dose of 100mg/kg i.e. 89.83% ($p$<0.01) as compared to Diclofenic sodium (standard drug) at a dose of 5mg/kg body weight i.e 63.63% ($p$<0.01). It is concluded that dry ripe fruit of *A. marmelos* possesses significant dual analgesic activities i.e. central and peripheral.

**Keywords**: *A. marmelos* dry ripe fruit, analgesic activity, rats and mice.

## INTRODUCTION

The world is rich in medicinal plants and humans can't survive on earth in the absence of this curative flora for long time, because of its key role. Presently throughout the world, herbs were use as principal ingredient in many medicines as people wants to treat illness with medicines that work in count with the body's own defense (Perumalsamy *et al.,* 1998). Generally it is believed that natural medicines are better and more risk-free or safer than unnatural or synthetic medicines (Brindha and Parvathi 2003; Dhankar *et al.,* 2011).

Instead of great achievement in synthetic medicines herbs are still playing a vital role regarding the human health as a preventive or curative agent because many green plants derived medicines are in use like quinine, morphine, paclitaxel, camptothecin, etoposide, mevastatin and artemisinin (Maridass and De Britto 2008). The reason is that along with the high cost of synthetic medicines they are also associated with some frequent side effects. In the light of this background searching of new and novel herbal drugs are still necessary to treat diseases because beside of their low cost and negligible side effects they have vast pharmacological and biological properties.

*Aegle marmelos* L. (Rutaceae) commonly known as Bael found in Asia and Africa (Hameed *et al.,* 2011). Due to its numerous curative properties including antipyretic, analgesic, anti-inflammatory, anti-dirroheal, hypoglycemic, laxative, antioxidant, hepatoprotective, anti-cancer, cardioprotective, anti-spermatogenic, antimicrobial, radioprotective, to treat peptic ulcer, to treat respiratory disorders etc it is one of the most useful plant (Sharma *et al.,* 2007; Supria *et al.,* 2011). Its photochemical studies reported that this plant contains more than 100 vital chemical constituents that are responsible for its medicinal properties (Maity *et al.,* 2009; Venkatesan *et al.,* 2009; Dama *et al.,* 2010). Phytochemical studies on *A. marmelos* reported the presence of certain vital constituents like tannins, reducing sugar, Phlobatannins, saponins, steroids, flavonoids, terpenoids, poly phenols, lignin, fat, oil, inulin, proteins, carbohydrates, cardiac glycoides, alkaloids that are responsible for its medicinal value. Another study reported that its fruit also contains water, sugar, protein, fiber, fat, calcium, phosphorus, potassium, iron, minerals and vitamins (A, $B_1$, $B_2$ and C), which are a good source of energy and also having antioxidant activity (Yadav *et al.,* 2011; Pandey and Mishra 2011; Sekar *et al.,* 2011). Almost its all parts including leaves, fruit, stem and roots are used for medicinal purpose but most useful, valuable and consumable part is fruit used in ripe and unripe form to treat chronic dirrohea, dysentery, as gastroprotective, luxatative, nutritive, tonic for heart and brain, analgesic, anti-inflammatory, antipyretic, in constipation and dyspepsia (Rao *et al.,* 2003).

Inspite of number of reported pharmacological activities on *A. marmelos* ripe fruit no research studies were present

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on its analgesic aspect therefore, we decided to find out its analgesic activity in scientific manner to boost the existing information on *A. marmelos*.

## MATERIAL AND METHOD

***Plant material***

The dry and ripe fruit of *Aegle marmelos.* L. was purchased from the local market and identified by the botanist of PCSIR Laboratories Complex; Karachi, the specimen voucher no: Am012/2012 was preserved in the herbarium of the pharmacology section of PCSIR Laboratories Complex; Karachi.

***Preparation of fruit's extract***

The dry and ripe fruit was cleaned of adulterant and then coarsely grounded including the pulp and the seeds present in the fruit. The coarse powdered material (2.25 kg) was soaked in the methanol: water (70:30) mixture at room temperature (23-25$^{o}$C) and capped in screw tight bottles with occasional shaking for 1 week. It was first filtered through muslin cloth and then through Whatman No.1 filters paper. This procedure was repeated three times, filtrate was pooled in glass bottle and then evaporated on rotary evaporator under reduced pressure (-760 mmHg) at 35-40$^{o}$C. This produced a dark brown thick semi solid crude extract of *A. marmelos* (600 gm). The crude extract was stored in amber glass bottle and preserved at -4$^{o}$C until use.

***Chemicals and drugs***

The chemicals used for this study include analytical grade of methanol, Diclofenac sodium (Platinum, Pakistan), Acetic acid (Merck, Germany).

***Animal selection***

Male Swiss albino mice (20-30g) and male Wistar rats (140-200g) were selected for the studies. The animals were reared at animal house of PCSIR Laboratories Complex; Karachi, and housed separately under strict observation for 3 weeks with free access to food and water. Food was withdrawn 12hr before the start of experiment. Those animals showing sluggish movement or any sign of illness were excluded from the study. The methods and procedures were approved by the committee of the ethical use of experimental animals of PCSIR Laboratories Complex Karachi. All the animals received humane care in compliance with the "National Research Council. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academy Press; 1996".

***Analgesic activity***

***Tail flick test***

The analgesic activity was conducted by Tail flick method according to the protocol as reported earlier by (Kumbhare and Sivakumar 2011; Yaqeen *et al*., 2013) on analgesiometer (UGO Basile, Italy). Male albino rats (140-200g) were divided into five groups (n=5). Group I, II and III served as test groups and received dose of 100, 250 and 500 mg/kg body weight per orally (p.o.) Am Cr respectively. Group IV received 5.0 mg/Kg body weight Diclofenac sodium p.o as standard drug while Group V i.e. control group received vehicle (distilled water) in the same volume by feeding cannula. The intensity of heat was adjusted at 5 ampere and the cut off time was 15 sec to avoid any damage or injury to exposed part of tail. The results were noted initially at 0.0 min (Tb) and at the intervals of 1.0, 2.0 and 3.0 hrs after the administration of test and standard drugs (Ta). Percentage analgesic activity was calculated as per formula shown below (Mwale and Masika 2010).

$$\%\ \text{of analgesic activity} = \frac{\text{Ta - Tb}}{\text{Tb}} \times 100$$

***Acetic acid-induced writhing test***

This test was conducted on Swiss albino mice. The animals were divided into five groups (n=5). Group I, II and III served as test groups and received doses of Am Cr i.e. 100, 250 and 500 mg/kg body weight p.o. respectively. Group IV received 5.0mg/Kg body weight Diclofenac sodium p.o. as standard drug while Group V serves as control group and received vehicle (distilled water) in the same volume by feeding cannula. The acetic acid abdominal constriction was induced by injecting 1% v/v acetic acid solution intraperitoneally in the volume of 0.1 mL/10g, 30 minutes after the test and standard drugs administration that causes abdominal muscle constriction together with an elongation of the body and stretching of the hind limbs. Mice were placed individually into cages and numbers of writhing movements displayed from 5 to 20 min after acetic acid injection were recorded. The activity was express in term of % inhibition of writhes produced by acetic acid (Kumbhare and Sivakumar 2011).

C-D/C × 100

Where:

C-Average number of writhing for control group

D-Average number of writhing of test and standard groups (Mwale and Masika 2010)

## STATISTICAL ANALYSIS

Results are expresses as mean ± SEM. Statistical analysis was carried out using ANOVA with Dunnett's multiple comparisons test using Graph Pad In Stat version 3.00, Graph Pad Software, CA, USA. The level of significance was considered at $p<0.05$.

## RESULTS

The experimental data obtained so far revealed that methanolic extract of dry ripe fruit of *A. marlmelos* possesses dual analgesic action i.e. central and peripheral. Am. Cr induced significant increase in latency period in dose dependent manner i.e. 65.38% at 100mg/kg,

395.37% ($p$<0.01) at 250mg/kg and 459.25% ($p$<0.01) at 500mg/kg body weight at 1hr after drug delivery while at 2hr effect was decreased i.e. 61.53% at 100mg/kg, 161.11% ($p$<0.01) at 250mg/kg and 165.74% ($p$<0.01) at 500mg/kg but elongation in latency period was again noted at 3 hr i.e. 106.15% at 100mg/kg dose, 251.85% ($p$<0.01) at 250mg/kg and 293.51% ($p$<0.01) at 500mg/kg respectively against tail flick test. The standard drug Diclofenac sodium at the dose of 5mg/kg continuously increased the latency period at 1, 2 and 3hr but less significant as compared to the test substance i.e. 79.43%, 113.08% and 222.42% ($p$<0.05) respectively (table 1, fig.1).

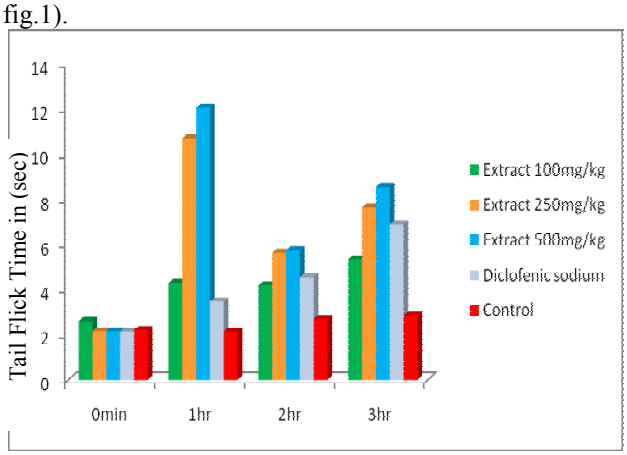


**Fig. 1**: Graph showing analgesic activity of test and standard drugs by tail flick test.

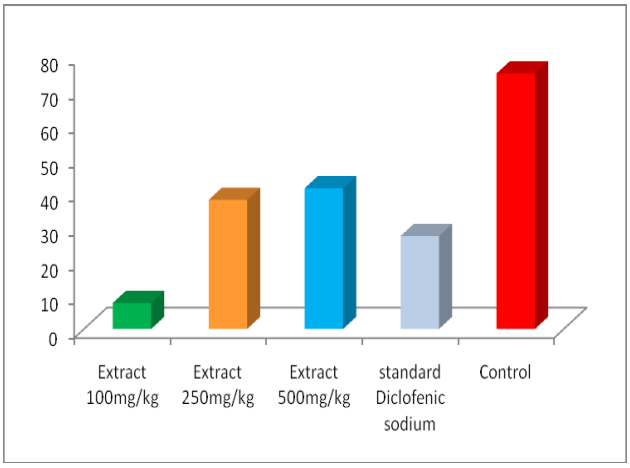


**Fig. 2**: Graph showing effects of test and standard drugs by acetic acid-induced writhing test.

Acetic acid-induced writhing test results showed that *A. marmelos* methanolic extract possesses significant analgesic activity at the dose of 100mg/kg body weight by inducing 89.83% inhibition ($p$<0.01) followed by 49.46% ($p$<0.01) at 250mg/kg body weight and 71.65% ($p$<0.01) at 500mg/kg body weight respectively. The standard group treated with diclofenac sodium (5mg/kg) also showed significant reduction in the number of writhes 63.63% ($p$<0.01) as compared to control group (table 2, figs. 2-3).

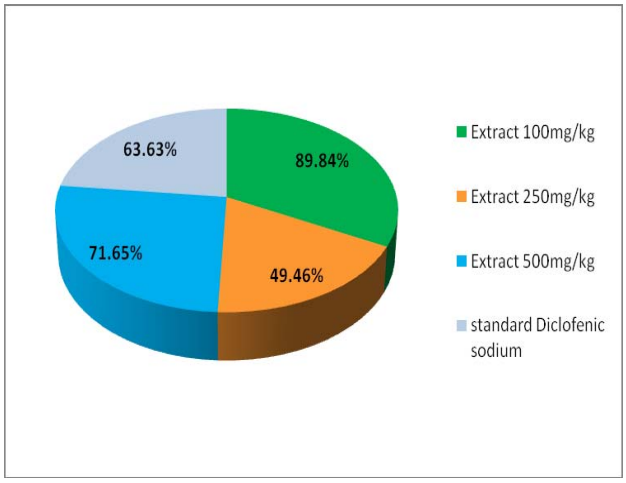


**Fig. 3**: Graph showing % of analgesic activity of test and standard drugs by acetic acid-induced writhing test.

## DISCUSSION

Pain is a defensive mechanism and can be defined as an acute discomfort which is possibly an alarm for some physical harm or disorder and may causes the person to react to remove the pain stimulus (Kanodia and Das 2009). To relief pain various types of painkillers, synthetic or herbal are available that act either peripherally or centrally. The methanolic extract of ripe fruit of *A. marmelos* exhibited both central and peripheral analgesic effects against radiant heat tail flick test and acetic acid-induced mouse writhing test respectively.

In this study results obtained from radiant heat tail flick test revealed that the test drug (Am. Cr) has stress tolerance capacity by enhancing the latency period in dose dependent manner. The maximum/potent analgesic effect of *A. marmelos* was observed at 1hour after drug administration. At the dose of 500mg/kg body weight Am. Cr showed more prominent effect 1 hr after the dose administration i.e. 459.25% ($p$<0.01) and 293.51% ($p$<0.01) at 3 hrs which was very significant followed by 250mg/kg which showed 395.37% ($p$<0.01) and 251.85% ($p$<0.01) at 1 and 3hrs respectively after drug delivery. The standard drug diclofenac sodium at the dose of 5mg/kg body weight exhibited maximum activity at 3hr after drug administration 222.42% ($p$<0.05) (table 1, fig.1).

The acetic acid-induced mouse writhing test has been used widely to qualify analgesic agent that have peripheral analgesic action (Neves *et al.*, 2007). It is known that the peripheral effects are due to the inhibition of pain-mediating autacoids like prostaglandins. The results of peripheral analgesic activity reveled that it reduces the number of abdominal contractions to a significant extent and maximum inhibition was observed at the dose of 100mg/kg body weight which showed more potent and highly significant effect i.e. 89.83% ($p$<0.01)

**Table 1**: Effect of methanolic extract of dry ripe fruit of *Aegle marmelos* L. on Tail flick latency period in rats

| Treatment | Dose (mg/kg) | Tail flick latency (in sec) at time (hr) | | | |
|---|---|---|---|---|---|
| | | 0 hr | 1 hr (% elongation) | 2 hr (% elongation) | 3 hr (% elongation) |
| Extract | 100mg/kg | 2.6±0.35 | 4.3±0.43 (65.38%) | 4.2±0.808 (61.53%) | 5.36±0.753 (106.15%) |
| Extract | 250mg/kg | 2.16±0.52 | 10.7±1.590** (395.37%) | 5.64±0.592** (161.11%) | 7.6±0.829** (251.85%) |
| Extract | 500mg/kg | 2.16±0.53 | 12.08±1.483** (459.25%) | 5.74±0.750** (165.74%) | 8.5±1.622** (293.51%) |
| Diclofenac sodium | 5 mg/kg | 2.14±0.74 | 3.48±0.124 (79.43%) | 4.56±0.103 (113.08%) | 6.9±0.184* (222.42%) |
| Control | – | 2.2±0.94 | 2.14±0.361 | 2.7±0.308 | 2.86±0.263 |

Data were statistically analyzed using one-way analysis of variance (ANOVA) and expressed as Mean ±SEM (n=5) followed by Dunnett's multiple comparisons test and critical differences between means were regarded significant at *p<0.05 and very significant at ** (*P*<0.01).

**Table 2**: Effect of methanolic extract of dry ripe fruit of *Aegle marmelos* L. on acetic acid induced writhing response in mice

| Treatment Groups | Dose (mg/kg) | No. of writhing | Inhibition (%) |
|---|---|---|---|
| Extract | 100 | 7.60±1.249 | 89.84%** |
| Extract | 250 | 37.80±5.083 | 49.46%** |
| Extract | 500 | 21.20±9.431 | 71.65%** |
| Diclofenac sodium | 5 | 27.20±5.748 | 63.63%** |
| Control | --- | 74.80±6.748 | ---- |

Data were statistically analyzed using one-way analysis of variance (ANOVA) and expressed as Mean ±SEM (n=5) followed by Dunnett's multiple comparisons test and critical differences between means were regarded significant at p<0.05 and very significant at ** (*P*<0.01).

followed by 49.46% ($p<0.01$) at 250mg/kg and 71.65% ($p<0.01$) at 500mg/kg respectively as compared to control group. The standard group treated with diclofenac sodium (5mg/kg) also showed significant reduction in the number of writhes 63.63% ($p<0.01$) (table 2, figs. 2 and 3).

Several studies reported that intra-peritoneal injection of acetic acid acts indirectly and produced pain through activation of chemosensitive nociceptors of irritation of visceral surface and lead to release of endogenous mediators, such as histamine, serotonin, bradykinins, PGE2 (prostaglandin E2) and PGE2α in peritoneal fluids as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs. Therefore, the results of the acetic acid induced writhing test strongly suggests that the mechanism of this extract may be linked partly to inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissues, reducing in PGE2 (Pal and Parwar 2011; Gupta *et al.,* 2011; Nirmul *et al.,* 2012; Ahmad *et al*., 2011). It is reported that the presence of flavonoids, steroids and tannins are responsible for analgesic activity (Kumbhare and Sivakumar 2011; Yaqeen *et al*., 2013). It is also well known that the enzyme prostaglandins are involved in pain perception and its synthetase is inhibited by flavonoids so it might be possible that the reduced availability of prostaglandins produce analgesic effects (Ahmed *et al.,* 2011).

According to Yadav *et al.,* 2011; Zulfiker *et al*., 2010; Joseph *et al.,* 2011 presence of tannin in extract is also responsible for analgesic activity and the quantity of tannin is more in ripe fruit as compare to unripe and half ripe fruit therefore it can be concluded that ripe fruit had more analgesic effects as compare to unripe fruit. According to Kumbhare and Sivakumar (2011) peripheral analgesic effect of drugs may be mediated through inhibition of cyclo-oxygenases and/or lipo oxygenases while central analgesic action may be mediated through inhibition of central pain receptors. All these studies support our results.

## CONCLUSION

Screening of methanolic extract of *A. marmelos* as an analgesic agent revealed that the extract might produce potent analgesic effect both peripherally and centrally. Results also showed that dry ripe fruit of *A. marmelos* produced more significant analgesia on the acetic acid-induced animal model than the tail flick radiant heat model and thus it appears that *A. marmelos* inhibits predominantly the peripheral pain mechanism which explains its common use as an analgesic agent in folk-lore system of treatment. Therefore on the basis of the dual analgesic action of *A. marmelos.* a useful herbal remedy may be prepared which can be used safely and effectively in pain disorders.

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